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Epigenetics in rheumatoid arthritis

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Abstract: PURPOSE OF REVIEW To give an overview of recently published articles addressing the role of epigenetic modifications in rheumatoid arthritis (RA). Here we focused on DNA methylation and posttranslational histone modifications. RECENT FINDINGS Recent studies attempted to link epigenetic modifications with genetic or environmental risk factors for RA. There is evidence that histone deacetylases confer effects of environmental triggers such as smoking, diet or therapy on expression levels of target genes. Additionally, disturbed methylation patterns and cell-type specific histone methylation marks were identified as potential mediators of genetic risk in RA. Altered methylome signatures were found in several cell types in RA, first of all RA synovial fibroblasts, and contribute to the intrinsic fibroblast activation. The reversal of DNA hypomethylation by inhibiting the polyamine recycling pathway was suggested as new epigenetic therapy in RA. Moreover, targeting epigenetic reader proteins, such as bromodomain proteins, emerged as a new field in drug development and the first studies underscored the potential of these drugs not only in malignant and inflammatory conditions but also in autoimmune diseases. SUMMARY Epigenetic factors represent a promising area to link genetics, regulation of gene expression and environmental risk factors.

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Epigenetics in rheumatoid arthritis

Kerstin Klein and Steffen Gay

Purpose of review

To give an overview of recently published articles addressing the role of epigenetic modifications in rheumatoid arthritis (RA). Here we focused on DNA methylation and posttranslational histone modifications.

Recent findings

Recent studies attempted to link epigenetic modifications with genetic or environmental risk factors for RA. There is evidence that histone deacetylases confer effects of environmental triggers such as smoking, diet or therapy on expression levels of target genes. Additionally, disturbed methylation patterns and cell-type specific histone methylation marks were identified as potential mediators of genetic risk in RA. Altered methylome signatures were found in several cell types in RA, first of all RA synovial fibroblasts, and contribute to the intrinsic fibroblast activation. The reversal of DNA hypomethylation by inhibiting the polyamine recycling pathway was suggested as new epigenetic therapy in RA. Moreover, targeting epigenetic reader proteins, such as bromodomain proteins, emerged as a new field in drug development and the first studies underscored the potential of these drugs not only in malignant and inflammatory conditions but also in autoimmune diseases.

Summary

Epigenetic factors represent a promising area to link genetics, regulation of gene expression and environmental risk factors.

Keywords

bromodomain protein, DNA methylation, histone, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease, characterized by chronic inflammation of the joints with severe pain and swelling, joint damage and disability, which leads ultimately to joint destruction and loss of function. RA can be divided into two major subsets based on the presence or absence of antibodies to citrullinated peptide antigens (ACPA) [1]. The best known genetic risk factor for ACPA⁺ RA is the presence of certain human leukocyte antigen (HLA) class II alleles. Several genome-wide association studies (GWAS) have identified genetic variants across different populations that confer risk to RA. However, these variants can explain only less than 20% of susceptibility in ACPA⁺ RA and even less in ACPA⁻ RA. The contribution of environmental triggers including smoking, the best known environmental risk factor for RA, is essential to RA pathogenesis [2,3]. The understanding of the interplay between immunity, life style as well as genetic and environmental factors is poor and is key to explaining the cause of RA. Epigenetic factors represent a promising area to link genetics and gene expression with disease risk [3].

EPIGENETIC FACTORS AS REGULATORS OF GENE EXPRESSION

The term 'epigenetics' was originally associated with heritable changes in gene activity that occur without alterations of the genetic code. Nowadays, the term is more often used to describe chromatin-related regulatory mechanisms that do not involve changes in the nucleotide sequence, regardless of whether such imprinting is strictly heritable [4]. Posttranslational histone modifications such as acetylation, methylation or phosphorylation marks among others are added to histone side chains or to cytosine residues in the DNA (DNA methylation) by epigenetic 'writer' proteins (Fig. 1). These epigenetic marks are recognized by 'reader' proteins that serve

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KEY POINTS

- Epigenetic modifications can serve as intermediaries of genetic risk and environmental factors in RA.
- Inhibition of the polyamine recycling pathway reverses the hypomethylation in rheumatoid arthritis synovial fibroblasts (RASf) and reduces the invasiveness of RASf *in vivo*.
- Targeting epigenetic reader proteins such as bromodomain and extraterminal proteins might become new therapies in autoimmune diseases.

as platforms and docking sites for effector proteins mediating fundamental processes such as transcription, DNA replication and recombination, DNA damage response and chromatin remodeling. Epigenetic 'eraser' proteins are capable of removing epigenetic marks. Understanding the intricate spatial and temporal interplay between different epigenetic modifications is one of the most challenging aspects in chromatin biology and at the beginning of exploration [4].

In addition to posttranslational histone modifications and DNA methylation, noncoding RNAs are additional factors that influence gene expression

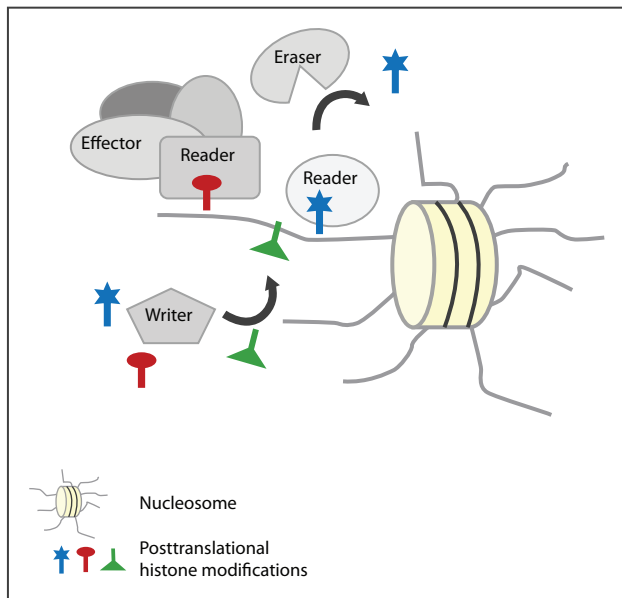


FIGURE 1. Epigenetic writer, reader and eraser proteins. Posttranslational histone modifications such as acetylation, methylation or phosphorylation marks are added to histone side chains by writer proteins and are recognized and get bound by reader proteins. Epigenetic reader proteins serve as a platform and docking site for effector proteins. Posttranslational histone modifications are removed by eraser proteins.

levels. Apart from microRNAs [5], which are small noncoding RNAs acting as destabilizers and repressors of translation, the functional role of other noncoding RNAs in RA has not been investigated yet. Long noncoding RNAs with a length of at least 200 nucleotides regulate gene expression levels by affecting the stability of mRNA, altering the translation efficiency, and functioning as precursors for mRNAs. Furthermore, long noncoding RNAs are capable of controlling the epigenetic state of particular genes by guiding chromatin-modifying complexes and other nuclear proteins to specific genomic loci to exert their effects [6]. Song *et al.* [7] have recently shown an altered pattern of long noncoding RNAs in peripheral blood mononuclear cells (PBMC) of RA patients compared with healthy controls but their impact on disease pathogenesis is at the beginning of investigation.

DNA METHYLATION

Methylation of DNA is carried out by the activity of DNA methyl transferases (DNMT), leading to the formation of 5-methylcytosine (5-mC). Most research so far has focused on the role of 5-mC in CpG rich regions, called CpG islands. They are enriched in promoters in the vicinity of transcriptional start sites and their methylation blocks the initiation of transcription and is associated with long-term gene silencing including X-chromosome inactivation [8].

An altered DNA methylome signature in synovial fibroblasts derived from RA and osteoarthritis patients, in PBMC from RA patients and healthy controls, as well as reduced amounts of 5-mC in synovial tissues of RA patients were described by different research groups [9,10,11[¶]]. Hypomethylated genes clustered in key pathways related to cell migration, including focal adhesion, cell adhesion, transendothelial migration and extracellular matrix interactions [11[¶]]. Karouzakis *et al.* [12[¶]] showed that increased levels of the polyamine-modulated factor 1-binding protein 1 (PMFBP1) and the spermidine/spermine N1-acetyltransferase (SSAT1) in rheumatoid arthritis synovial fibroblasts (RASf) enhanced the catabolism and recycling of polyamines. The authors suggested that the high consumption of S-adenosyl methionine (SAM), the methyl donor during DNA methylation via this pathway as an important factor contributing to the global DNA hypomethylation in RASf [12[¶]]. In a follow-up study, Neidhart *et al.* [13^{¶¶}] recently suggested a new therapeutic concept for inhibiting the ongoing joint destruction in RA based on the inhibition of the polyamine recycling pathway by using diminazene

aceturate, an inhibitor of the SSAT-1, alone, or in combination with SAM/L-methionine. The authors showed that SSAT-1 inhibition reduced the adhesion abilities and expression levels of matrix metalloproteinase (MMP) 1 in RASF as well as the invasiveness of RASF that were coimplanted with cartilage into the severe combined immunodeficiency mouse model [13²²]. This would be the first therapy targeting RASF.

Besides global changes in DNA methylation, promoters of specific genes were shown to be hypomethylated or hypermethylated in RA in different cell types [14,15,16²,17]. The methylation of a single CpG site in the promoter of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) in regulatory T cells (Treg) of RA patients was shown to result in impaired binding of the transcription factor nuclear factor of activated T cells 2 (NFAT2) leading to decreased expression levels of CTLA-4 in RA patients compared with healthy controls. As a consequence, Treg were unable to activate the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase and therefore failed to activate the immune modulatory kynurenine pathway [16²]. These results implicate that small epigenetic changes can impact the function of a whole cell type in RA, in this case Treg.

HISTONE ACETYLATION

Histone acetylation and deacetylation are executed by the activity of two opposing enzyme families: histone acetylases (HAT) and histone deacetylases (HDAC). HDAC exert the epigenetic control of transcriptional activity by removing negatively charged acetyl groups from lysine residues of histones leading to the condensation of chromatin and reducing the accessibility for transcription factors. The human genome contains 18 genes encoding for HDAC that are classified into four groups based on structural and functional similarities. Whereas class I HDAC (HDAC 1, 2, 3, 8) are widely expressed and present in nuclei, class II HDAC (HDAC 4–7, 9) exhibit a more limited expression pattern and are able to shuttle between the nucleus and the cytoplasm. Sirtuins (SIRT 1–7) are members of class III HDAC and require NAD⁺ for their enzymatic activity. HDAC11 is the sole member of class IV HDAC [18]. In addition to dietary factors that are known to affect HDAC activity [19], treatment of RA patients with antitumor necrosis factor (TNF) α therapy was shown to increase the HAT/HDAC ratio in nuclear extracts of PBMC, whereas rituximab increased nuclear activity of both HAT and HDAC [20]. Recently, direct effects of smoking on changes in gene expression levels in joints were described

[21²²,22²] and SIRT6 was identified as one of the mediators of smoke-induced changes in RASF. Engler *et al.* [22²] showed that SIRT6 levels were increased in synovial tissues of smokers and SIRT6 levels correlated with the disease duration in smokers but not in nonsmokers. They showed that the up-regulation of SIRT6 in RASF under exposure to cigarette-smoke extract or TNF α functions as counterregulatory mechanism attenuating the production of MMP1 [22²]. These findings were in line with a previous report revealing an anti-inflammatory and antidestructive role of SIRT6 in RASF *in vitro* and in the collagen-induced arthritis (CIA) mouse model *in vivo* [23²²]. On the contrary, SIRT1, expression levels of which are increased in synovial tissues and synovial cells of RA patients, was shown to have proinflammatory properties and contributes to the apoptosis-resistant phenotype seen in RASF [24]. These reciprocal functions of SIRT1 and SIRT6 in RASF underscore the importance of performing functional studies on individual members of HDAC families.

Different HDAC inhibitors (Table 1), which mostly target several members of HDAC, were described to have beneficial effects *in vitro* and *in vivo* in RA [17,25–31]. However, many of the observed effects of HDAC inhibitors were shown to be chromatin-independent and nonepigenetic (reviewed in [17]). Further studies [29–31] (Table 1) have been published within the last 2 years using new and even more potent HDAC inhibitors. Ahmed *et al.* [29] showed that Largazole (LAR), a marine-derived class I HDAC inhibitor, suppressed the TNF α -induced expression of intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) in RASF, whereas the TNF α -induced MMP2 activity was reduced. Furthermore, the authors showed that LAR modulated expression levels of HDAC1, HDAC5 and HDAC6 and indicated a role of HDAC6 in the LAR-induced changes of ICAM-1 and VCAM-1 expression levels. These results again point to the need for understanding the exact role of different HDAC enzymes in the RA pathogenesis in order to develop highly effective HDAC inhibitors for the treatment of RA [29].

EPIGENETIC MECHANISMS AS AN INTERMEDIARY OF GENETIC RISK

Recent research efforts tried to integrate epigenetic mechanisms into genetic susceptibility loci as causes of the disease [32²²,33²²]. Liu *et al.* [32²²] performed a genome-scale methylation and single nucleotide polymorphism (SNP) analysis in leukocytes derived from ACPA⁺ patients. The authors identified nine

Table 1. Histone deacetylases inhibitors that were recently experimentally used in rheumatoid arthritis

Target(s)	Drug	Structural class	Findings	References
HDAC1, HDAC3, Class I and Class II HDAC	MS-275 (Entinostat), Suberoylanilidehydroxamic acid (SAHA, Vorinostat)	Benzamide, Hydroxamic acid	Suppressed LPS-induced nuclear accumulation of NF- κ B p65 in synovial fibroblastic E11 cells and THP-1 monocytes	[25]
HDAC3	MI192	Benzamide	Reduced LPS-induced interleukin-6 production in PBMC from RA patients	[26]
Class I and Class II HDAC	TSA	Hydroxamic acid	Reduced interleukin-6 mRNA stability in macrophages and RASF	[27]
Class I (HDAC 1,2,3,8) and Class II (HDAC 4,5,6,7,9,10) HDAC	ITF2357 (Givinostat)	Hydroxamic acid		
Class III (Sirtuins; SIRT1–7)	TSA	Hydroxamic acid	Reduced TNF α induced	[28]
	Nicotinamide	Amide of vitamin B ₃	Interleukin-6 expression and reduced LPS induced interleukin-6 and TNF α expression in macrophages	
Class I HDAC and HDAC6	Largazole (LAR)	Cyclic peptide	Induction of apoptosis in macrophages	
			Enhanced TNF α -induced ICAM-1 and VCAM-1 expression in RASF; inhibited TNF α -induced MMP2 activity; modulated expression levels of Class II HDAC	[29]
Class I HDAC and HDAC6	MPT0G009	Hydroxamic acid	Reduced PGE2 and interleukin-6 secretion in RASF; reduced osteoclast formation; reduced paw swelling and arthritis scores in adjuvant-induced arthritis rats	[30]
HDAC1 (others?)	S-[E]-3-(1-(1-(benzo[dioxazol-2-yl)-2-methylpropyl]-1H-1,2,3-triazol-4-yl)-N-hydroxyacrylamide (NK-HDAC1)	Hydroxamic acid	Reduced proliferation rates of RASF and suppressed TNF α -induced interleukin-6 and MMP3 secretion; increased apoptosis of synoviocytes and inhibited disease progression in CIA mice	[31]

CIA, collagen-induced arthritis; HDAC, histone deacetylases; ICAM, intracellular adhesion molecule; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; NF- κ B, nuclear factor-kappaB; PBMC, peripheral blood mononuclear cells; RASF, rheumatoid arthritis synovial fibroblasts; THP, human leukemic cell line; TNF, tumor necrosis factor; TSA, Trichostatin A; VCAM, vascular adhesion molecule.

clusters of differentially methylated signatures in the MHC region and one outside on the same chromosome that likely mediate the genetic risk for RA [32²²]. Furthermore, in a three-stage trans-ethnic meta-analysis of GWAS data, an enrichment of non-MHC risk loci was assessed in epigenetic chromatin marks [33²³]. Histone 3 lysine 4 trimethylation (H3K4me3), a mark that highlights active promoters and enhancers, was previously shown to be the histone mark that is most phenotypically cell-type specific [34]. RA risk loci in biological candidate genes overlapped with H3K4me3 peaks in immune-related cells, especially in Treg [33²³].

OTHER POSTTRANSLATIONAL HISTONE MODIFICATIONS

Only a few studies analyzed the role of other posttranslational histone modifications than acetylation in RA (reviewed in [17]). Recently, the first study on histone phosphorylation in arthritis was published. The authors identified an overexpression of Aurora kinases A (AURKA) and B (AURKB) in mononuclear cells derived from proteoglycan-induced arthritis (PGIA) mice and PBMC derived from treatment naive RA patients compared with healthy controls by screening 84 known chromatin-modifying enzymes. Increased AURKA and AURKB levels in B cells derived from PGIA mice correlated with elevated levels of phosphorylated histone 3. Treatment with a pan-aurora kinase inhibitor promoted B-cell apoptosis and attenuated inflammatory reactions in arthritic mice [35²⁴].

TARGETING EPIGENETIC READER PROTEINS

In recent years, much effort was put into the development of small molecule inhibitors of the bromodomain and extraterminal (BET) family (BRD2, BRD3, BRD4, BRDT) of bromodomain proteins. BET proteins are readers of the ϵ -N-acetylation of lysine residues (Kac) on histone tails, a modification that is associated with an open chromatin architecture and transcriptional activation [36]. First clinical trials using the BET inhibitors I-BET762 for the treatment of nuclear protein in testis (NUT) midline carcinomas, OTX015 for the treatment of hematological malignancies and CPI-0610 for the treatment of lymphomas have started recently (reviewed in [37]), underscoring the potential of this class of inhibitors. However, BET inhibition as a point of therapeutic intervention also became of interest in autoimmune conditions because of the strong anti-inflammatory properties of BET inhibitors [38,39]. A first hint for an association of BET proteins with RA came from a study by Mahdi *et al.* [40] in which specific interactions between genotype, smoking and autoimmunity to citrullinated α -enolase were analyzed. Three SNPs in the BRD2 locus were found to be associated with a subset of RA patients positive for citrullinated α -enolase peptide 1 and cyclic citrullinated peptides, independently of the *HLA-DRB1* shared epitope alleles [40]. In addition, a SNP in the locus of another epigenetic reader protein, namely BRD1 (BRPF2) was shown to be protective in joint damage progression in stage I of a GWAS in ACPA⁺ RA patients [41]. Recently, therapeutic dosing of the BET inhibitor JQ1 was proven to be

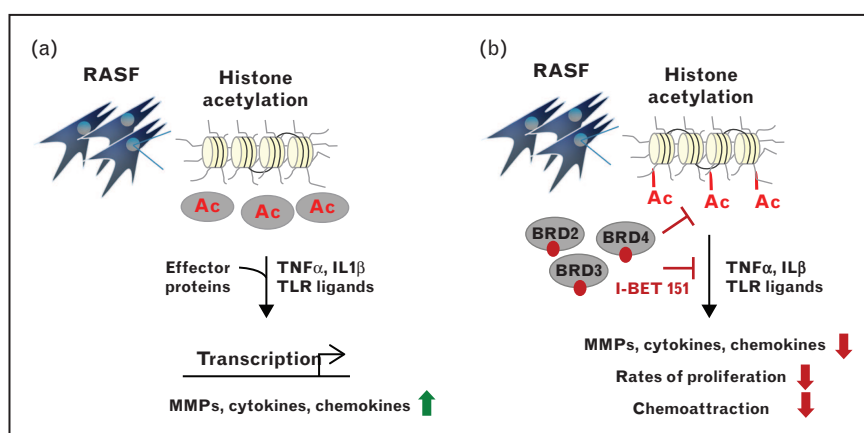


FIGURE 2. Targeting epigenetic reader proteins with I-BET151. (a) Acetylated side chains of histones are recognized by the epigenetic reader proteins BRD2, BRD3 and BRD4, leading to the recruitment of effector proteins and the activation of transcription. (b) I-BET151 is a histone mimic that prevents BRD2, BRD3, and BRD4 from binding to acetylated histone side chains, suppresses the transcription of matrix degrading enzymes, inflammatory cytokines and chemokines after stimulation with TNF α , interleukin-1 β or Toll-like receptor (TLR) ligands and reduces proliferation rates and the chemoattractive potential of RASF. BET, bromodomain and extraterminal; RASF, rheumatoid arthritis synovial fibroblasts; TNF, tumor necrosis factor.

efficacious in two mouse models of autoimmunity, the models for CIA and for experimental autoimmune encephalomyelitis (EAE); BET inhibition suppressed the differentiation and activation of Th17 cells [42²²]. Furthermore, we have shown that the inhibitor I-BET151 suppressed the expression of a broad range of inflammatory mediators and matrix degrading enzymes in RASF in the presence of cytokines or Toll-like receptor (TLR) ligands (Fig. 2). Furthermore, I-BET151 reduced proliferation rates of RASF and chemotactic properties towards PBMC (Klein *et al.*, unpublished). However, the individual roles of BET proteins in mediating effects of different BET inhibitors in inflammatory and autoimmune conditions has hardly been investigated yet.

Besides targeting bromodomain proteins [37], readers of other epigenetic marks including selected members of methyl-lysine binding site readers were predicted to have good drugability and first inhibitors are in development [43].

CONCLUSION

The comprehension of the role of epigenetic factors contributing to the pathogenesis of RA has increased in recent years. A future challenge is to reveal the interplay between different epigenetic modifications and to implement more functional studies in order to understand underlying mechanisms of epigenetic factors in different cell types contributing to RA pathogenesis.

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Conflicts of interest

None.

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